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REVIEW



## Targeting metabotropic glutamate receptors in the treatment of epilepsy: rationale and current status

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### ABSTRACT

**Introduction:** Several drugs targeting the GABAergic system are used in the treatment of epilepsy, but only one drug targeting glutamate receptors is on the market. This is surprising because an imbalance between excitatory and inhibitory neurotransmission lies at the core of the pathophysiology of epilepsy. One possible explanation is that drug development has been directed towards the synthesis of molecules that inhibit the activity of ionotropic glutamate receptors. These receptors mediate fast excitatory synaptic transmission in the central nervous system (CNS) and their blockade may cause severe adverse effects such as sedation, cognitive impairment, and psychotomimetic effects. Metabotropic glutamate (mGlu) receptors are more promising drug targets because these receptors modulate synaptic transmission rather than mediate it.

**Areas covered:** We review the current evidence that links mGlu receptor subtypes to the pathophysiology and experimental treatment of convulsive and absence seizures.

**Expert opinion:** While mGlu<sub>5</sub> receptor negative allosteric modulators have the potential to be protective against convulsive seizures and hyperactivity-induced neurodegeneration, drugs that enhance mGlu<sub>5</sub> and mGlu<sub>7</sub> receptor function may have beneficial effects in the treatment of absence epilepsy. Evidence related to the other mGlu receptor subtypes is more fragmentary; further investigations are required for an improved understanding of their role in the generation and propagation of seizures.

### ARTICLE HISTORY

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Metabotropic glutamate receptors; epilepsy; convulsive seizures; absence seizures

### 1. Introduction

An imbalance between excitatory and inhibitory neurotransmission is one of the key mechanisms in the development of epileptic seizures, and this provides the rationale for the use of drugs that enhance inhibition by increasing extracellular GABA concentration (e.g., valproate, tiagabine, vigabatrine) or activating GABA<sub>A</sub> receptors (e.g., benzodiazepines). On the other side of the coin, only one drug that reduces excitation by specifically targeting glutamate receptors, the  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor antagonist perampanel, is currently used in the treatment of focal epilepsy [1]. This is quite surprising if one considers the higher density of excitatory pyramidal neurons with respect to inhibitory GABAergic interneurons in the cerebral cortex and hippocampus (e.g., about 85% vs. 15% of the overall neuronal population in particular regions of the cerebral cortex, respectively). Glutamate receptors are divided into two major superfamilies: (i) ionotropic (iGlu) receptors, which form membrane cation channels; and (ii) metabotropic (mGlu) receptors, which are coupled to effectors via G proteins. iGlu receptors include AMPA, NMDA (N-methyl-D-aspartate), and kainate receptors, and they are all formed by the heteromeric assembly of four subunits, which determine the ion selectivity and kinetic properties of the ion channels. AMPA-gated ion channels are selectively permeable

to Na<sup>+</sup> ions at most of the excitatory synapses because of the presence of the GluA2 subunit, which prevents Ca<sup>2+</sup> influx through the channel. In contrast, NMDA receptors are highly permeable to Ca<sup>2+</sup> ions, but they are inhibited by physiological concentrations of extracellular Mg<sup>2+</sup> [2]. Membrane depolarization removes the Mg<sup>2+</sup> blockade of the NMDA-gated ion channel, thereby allowing a large influx of extracellular Ca<sup>2+</sup> in neurons when NMDA receptors are activated by glutamate in the presence of the co-agonist, glycine [2,3]. Activation of NMDA receptors is a key event in the induction of activity-dependent synaptic plasticity (i.e., long-term potentiation and long-term depression of excitatory synaptic transmission) [4], and is also involved in the induction of electrical kindling [5,6], which is a model of epileptogenesis, i.e. gradual process by which a normal brain is biased towards the generation of epilepsy. One of the reasons why drugs that primarily block iGlu receptors are not currently used in the treatment of epilepsy (with the notable exception of perampanel) is that iGlu receptors mediate fast excitatory synaptic transmission, and chronic pharmacological blockade of these receptors may cause a sustained depression of synaptic transmission and an impairment of activity-dependent synaptic plasticity resulting in cognitive dysfunction. In addition, pharmacological inhibition of NMDA receptors may produce psychotomimetic effects, as typically observed with the slow NMDA channel blockers, ketamine and phencyclidine [7].

**Article Highlights**

- There is a need for new drugs for the treatment of epilepsy.
- Metabotropic glutamate receptors are candidate drug targets for the treatment of epilepsy because they modulate excitatory and inhibitory neurotransmission.
- Positive allosteric modulators of mGlu<sub>5</sub> and mGlu<sub>7</sub> receptors hold promise for the treatment of epileptic syndromes characterized by absence seizures.
- Drugs that activate mGlu<sub>2</sub> receptors or inhibit mGlu<sub>5</sub> receptors are potentially effective in the treatment of convulsive seizures.
- The study of mGlu receptors in the process of epileptogenesis is in its infancy.

This box summarizes the key points contained in the article.

As opposed to iGlu receptors, mGlu receptors do not mediate but rather ‘modulate’ synaptic transmission acting at different levels of the tripartite synapse formed by the junction of axon terminals, dendritic spines, and astrocytes [8,9]. This shifts attention to mGlu receptors as candidate drug targets in the experimental treatment of epilepsy.

## 2. Metabotropic glutamate receptors and epilepsy

mGlu receptors are G protein-coupled receptors activated by glutamate and are involved in the modulation of synaptic transmission. They are mainly expressed in the central nervous system (CNS) and consist of eight subtypes divided into three groups based on amino acid sequence, pharmacological properties, and signaling transduction pathways [10]. Specifically, group I comprises mGlu<sub>1</sub> and mGlu<sub>5</sub> receptors, which are expressed primarily at postsynaptic sites and are coupled to G<sub>q</sub>/G<sub>11</sub> proteins associated with stimulation of the phospholipase C pathway. Their activation stimulates the hydrolysis of phosphatidylinositol-4,5-bisphosphate, with the formation of inositol-1,4,5-trisphosphate (InsP<sub>3</sub>) and diacylglycerol (DAG). InsP<sub>3</sub> and DAG enhance intracellular Ca<sup>2+</sup> release and activate protein kinase C, respectively. Group II comprises mGlu<sub>2</sub> and mGlu<sub>3</sub> receptors coupled to G<sub>i</sub>/G<sub>o</sub> proteins, which transduce the signal via inhibition of adenylyl cyclase and the modulation of Ca<sup>2+</sup> and K<sup>+</sup> channels [11]. mGlu<sub>2</sub> receptors are mainly localized in nerve endings, where they negatively modulate neurotransmitter release. mGlu<sub>3</sub> receptors share the same presynaptic localization, but, in addition, are also found in postsynaptic elements, where they functionally interact with mGlu<sub>5</sub> receptors [12]. mGlu<sub>3</sub> receptors are also expressed by astrocytes and microglia, where they regulate the production of neurotrophic factors and cytokines [9]. Finally, group III comprises mGlu<sub>4</sub>, mGlu<sub>6</sub>, mGlu<sub>7</sub>, and mGlu<sub>8</sub> receptors, which are coupled to G<sub>i/o</sub> proteins, and are expressed in presynaptic terminals where they inhibit neurotransmitter release [13].

mGlu receptors form constitutive dimers composed of two subunits linked by a disulfide bridge [14]. Each subunit contains an extracellular orthosteric glutamate binding site (Venus Flytrap domain, VFTD [15]), and allosteric binding sites located within the seven transmembrane domains (7TMD) [16].

mGlu receptors are proposed as potential therapeutic targets for the treatment of neurologic and psychiatric disorders

and, thus, several compounds have been developed as subtype-selective mGlu receptor ligands [9,17].

Almost 30% of epileptic patients are resistant to current pharmacological treatments [18], which include (i) inhibitors of voltage-sensitive sodium channels, such as phenytoin, carbamazepine, oxcarbazepine, lamotrigine, and lacosamide; (ii) GABA-mimetic drugs, such as benzodiazepines, barbiturates, tiagabine, and vigabatrine; (iii) drugs interacting with the SV2A synaptic vesicle protein, such as levetiracetam and brivaracetam; (iv) inhibitors of T-type voltage-sensitive calcium channels, such as ethosuximide; (v) inhibitors of the  $\alpha_2\beta_1$  subunit of voltage-sensitive calcium channels, such as gabapentin and pregabalin; and (vi) drugs showing pleiotropic mechanisms, such as valproate, topiramate, zonisamide, and felbamate. In addition, some conventional antiepileptic drugs such as phenytoin and carbamazepine have a narrow therapeutic margin and require therapeutic drug monitoring [19]. Thus, new anti-epileptic drugs to ameliorate current therapies and to treat the drug-resistant form of epilepsies are badly needed [20]. Owing to their modulatory function, mGlu receptors have been implicated in the control of epileptic seizures [21], and in mechanisms of epileptogenesis and hyperactivity-induced neuronal damage [22,23].

Several selective agonists and antagonists of individual mGlu receptor subtypes have been developed as valuable tools to probe the role of mGlu receptors in physiology and pathology, and some of these compounds have been, or are currently being evaluated in clinical studies [24].

mGlu receptors could play a role in ictogenesis by increasing or decreasing the effects caused by an excessive activation of iGlu receptors [25], and some subtype-selective ligands might improve the therapeutic effect of antiepileptic drugs by modulating either excitatory or inhibitory neurotransmission [26]. The role of mGlu receptors in the pathophysiology and treatment of epilepsy has been the subject of numerous review articles in the last two decades [27–33].

The recent development of selective ligands of individual mGlu receptor subtypes and their use in experimental animal models of convulsive and absence seizures gave us the impetus to further discuss the topic and comment on the potential use of these compounds in the treatment of epilepsy.

### 2.1. Group I mGlu receptors and convulsive seizures

A large body of evidence has shown that mGlu<sub>5</sub> receptors are up-regulated in tissue specimens from patients suffering from temporal lobe epilepsy, as well as in the rodent hippocampus after induction of limbic seizures. Eleonora Aronica and her Associates were the first to show an increased expression of mGlu<sub>5</sub> (and mGlu<sub>3</sub>) receptors in hippocampal astrocytes in a rat model of temporal lobe epilepsy [34]. These findings laid the groundwork for the study of mGlu receptor expression in the hippocampus of patients suffering from mesial temporal lobe epilepsy (TLE). Tang et al. [35] found increases in mGlu<sub>1</sub> and mGlu<sub>5</sub> receptor immunoreactivity in the dentate gyrus and CA1 region of TLE patients. While mGlu<sub>1</sub> immunoreactivity was exclusively localized in postsynaptic elements, mGlu<sub>5</sub> receptor immunoreactivity was detected in both pre- and postsynaptic elements, as well as

in astrocytes. Similarly, Blümcke et al. [36] observed an increased expression of mGlu<sub>1</sub> receptors in the dentate gyrus of epileptic human and rat hippocampus. The up-regulation of mGlu<sub>5</sub> receptors was confirmed in more recent studies examining hippocampal tissue specimens of drug-resistant patients with TLE. The increase in mGlu<sub>5</sub> receptor expression was more prominent in patients with Ammon's horn sclerosis, where it correlated with mossy fiber sprouting [37,38]. One possible interpretation of these data is that the increased expression of mGlu<sub>5</sub> receptors contributes to mechanisms of maladaptive neuronal plasticity that sustain seizures associated with TLE or, alternatively, might represent a defensive mechanism of postsynaptic adaptation that tries to control over-excitation [38].

Pharmacological blockade of either mGlu<sub>1</sub> or mGlu<sub>5</sub> receptors is protective in models of convulsive seizures, suggesting that the overall function of group-I mGlu receptors is to increase excitability in neuronal circuits involved in the generation of convulsive seizures. Brian Meldrum and his Associates were pioneers in the study of group-I mGlu receptor antagonists in models of convulsive seizures. Intracerebroventricular (i.c.v.) injection of the selective orthosteric mGlu<sub>1</sub> receptor antagonists, LY367385 ((+)-2-methyl-4-carboxyphenylglycine) and AIDA ((RS)-1-aminodan-1,5-dicarboxylic acid), was protective against a generalized type of epilepsy with audiogenic seizures (DBA/2 mice). These two compounds could also reduce sound-induced clonic seizures when they were locally infused in the inferior colliculus of genetically epilepsy prone rats (GEPRs) [39]. Systemic administration of 2-methyl-6-(phenylethynyl)-pyridine (MPEP) or (E)-6-methyl-2-styryl-pyridine (SIB-1893), which behave as potent and selective mGlu<sub>5</sub> receptor negative allosteric modulators (NAMs), was also protective against sound-induced seizures in DBA/2 mice [40]. NAMs interact with allosteric sites of mGlu receptors inhibiting receptor activation regardless of the ambient concentrations of glutamate. The anti-seizure activity of mGlu<sub>5</sub> receptor blockers was less prominent in the kindling model of epilepsy. Acute treatment with MPEP could reduce seizures and afterdischarge durations in kindled rats but only when combined with subprotective doses of valproate or phenobarbital [41]. As expected, molecules that antagonize both mGlu<sub>1</sub> and mGlu<sub>5</sub> receptors, such as LY393053 (2-amino-2-(3-cis and trans-carboxycyclobutyl)-3-(9H-thioxanthen-9-yl) propionic acid), LY339764 ((R,S)-2-amino-2-(4-carboxycyclobutyl-3-(9-xanthen-9-yl) propanoic acid), LY367335 (2-Amino-2-(3-cis and trans-carboxycyclobutyl)-3-(9H-xanthen-9-yl) propionic acid), LY367366 ((R,S)-2-amino-2-(4-carboxyphenyl)-3-(9H-thioxanthen-9-yl) propanoic acid), and LY339840 ((R,S)-2-methyl-3-hydroxy-4-carboxyphenylglycine), were highly protective against limbic seizures induced by i.c.v. injection of the mixed mGlu<sub>1/5</sub> receptor agonist, 3,5-dihydroxyphenylglycine (DHPG) [42]. Zavala-Tecuapetla et al. [43] examined the effect of the selective mGlu<sub>5</sub> receptor NAM, 3-(2-methyl-4-thiazolyl)ethynylpyridine (MTEP), in an in vivo model of complex partial seizures based on repeated electrical stimulations of the dorsal hippocampus in rats. Treatment with MTEP suppressed epileptic after discharges in 12- and 18-day-old rats, but not in 25-day-old rats. Systemic treatment with the mGlu<sub>1</sub> receptor orthosteric antagonist, AIDA, or with the mGlu<sub>5</sub> receptor NAM, MPEP, was highly protective against audiogenic seizures in a genetic rat model of reflex epilepsy [44]. A decrease

in seizure threshold for pentylenetetrazole (PTZ) during diazepam withdrawal was suppressed by i.c.v. pretreatment with the group I mGlu receptor antagonist, (S)-4-carboxyphenylglycine ((S)-4CPG) [45]. In contrast, treatment with mGlu<sub>1</sub> or mGlu<sub>5</sub> receptor antagonists (EMQMCM ([3-ethyl-2-methyl-quinolin-6-yl-(4-methoxy-cyclohexyl)-methanone methanesulfonate]) and MPEP, respectively), did not produce robust anticonvulsant effects in the 6-Hz test and amygdaloid kindling, two models for difficult-to-treat secondarily generalized focal epilepsies [46]. Taken together, these data suggest that mGlu<sub>1</sub> and mGlu<sub>5</sub> receptors blockers are more effective in models of generalized convulsive epilepsies than in models of partial epilepsies. While all these data clearly demonstrate that endogenous activation of either mGlu<sub>1</sub> or mGlu<sub>5</sub> receptors facilitates the generation of convulsive seizures, genetic deletion of mGlu<sub>5</sub> receptors did not protect against chemically induced seizures in mice [47]. In the same study, systemic treatment with MTEP did not reduce NMDA-induced seizures [47]. These findings do not support the hypothesis of a pro-convulsant activity of mGlu<sub>5</sub> receptors in mice. Again, the complex role of mGlu receptors in the modulation of synaptic transmission may contribute to provide an explanation for these conflicting findings.

## 2.2. Group-I mGlu receptors in models of absence epilepsy

Expression and function of mGlu<sub>1</sub> and mGlu<sub>5</sub> receptors have been extensively investigated in a variety of experimental animal models of absence epilepsy. Absence seizures are non-convulsive seizures associated with bilateral symmetrical so-called 'generalized' spike-and-wave discharges (SWDs) at the electroencephalogram (EEG), which are generated by pathological oscillations of a cortico-thalamo-cortical neuronal network formed by the somatosensory cortex, the ventrobasal thalamic nuclei, and the reticular thalamic nuclei [48]. Both genetic epilepsy models and acute pharmacological seizure models of absence epilepsy are available. Rats of the Wistar Albino Glaxo/Rijswijk (WAG/Rij) strain represent a genetic absence model, and they develop spontaneous absence seizures after 2–3 months of age [49,50]. We have found that expression and function of mGlu<sub>1</sub> receptors were reduced in the thalamus of symptomatic (8-month-old) WAG/Rij rats compared to both age-matched non-epileptic control rats and young pre-symptomatic WAG/Rij rats [51]. These findings were confirmed by Karimzadeh et al. [52], who also found a reduction of mGlu<sub>1</sub> receptor mRNA and protein levels in the thalamus of symptomatic WAG/Rij rats. Expression of mGlu<sub>1</sub> receptors was also reduced in the hippocampus of double mutant (zi/zi, tm/tm) spontaneously epileptic rats, which exhibit both absence-like seizures and tonic convulsions [53]. Moving from the reduced mGlu<sub>1</sub> receptor function found in the thalamus of symptomatic WAG/Rij rats, we have hypothesized that pharmacological enhancement of mGlu<sub>1</sub> receptors could display 'therapeutic activity' against absence seizures. We tested this hypothesis using compound RO0711401 (9H-xanthene-9-carboxylic acid (4-trifluoromethyl-oxazol-2-yl) amide), which behaves as a selective and brain-permeant mGlu<sub>1</sub> receptor positive allosteric modulator (PAM). mGlu receptor PAMs bind to a receptor site different than the glutamate recognition site (usually in the 7-TM domains) and amplify receptor response to glutamate acting in an activity-dependent manner. Systemic administration of low doses



of RO0711401 (3 or 10 mg/kg) significantly reduced the frequency of occurrence of SWDs in WAG/Rij rats, whereas higher doses (30 mg/kg) reduced both the frequency and the mean duration of SWDs. In contrast, administration of the mGlu<sub>1</sub> receptor NAM, JNJ16259685 ((3,4-dihydro-2H-pyranol[2,3-b]quinolin-7-yl) (cis-4-ethoxycyclohexyl)methanone)) increased the frequency of SWD occurrence [51]. Similar data were obtained by Karimzadeh et al. [52], who found a reduction in the mean duration of SWDs after intrathalamic infusion of the mGlu<sub>1/5</sub> receptor agonist, DHPG, and an increase in the duration and amplitude of SWDs after infusion of the mGlu<sub>1</sub> antagonist, LY367385, in WAG/Rij rats. These findings suggest that mGlu<sub>1</sub> receptors are candidate drug targets for the treatment of absence epilepsy. However, the evidence that the anti-absence activity of RO0711401 was lost after the first two days of repeated administration [54] suggests the development of tolerance, which might be a major drawback in the therapeutic use of mGlu<sub>1</sub> receptor PAMs. Our studies in WAG/Rij rats indicate also that mGlu<sub>5</sub> receptors are a more promising candidate drug target for the treatment of absence epilepsy. Similar to mGlu<sub>1</sub> receptors, the expression and function of mGlu<sub>5</sub> were reduced in the thalamus of WAG/Rij rats. This reduction was also observed in pre-symptomatic WAG/Rij rats, suggesting that mGlu<sub>5</sub> receptors lie at the core of the generation of absence seizures in this strain of rats [55]. Systemic administration of the mGlu<sub>5</sub> receptor PAM, VU0360172 ((N-cyclobutyl-6-[2-(3-(fluorophenyl) ethynyl)] pyridine-3-carboxamide)), reduced both the frequency of occurrence and mean duration of SWDs in WAG/Rij rats, and its action was antagonized by the mGlu<sub>5</sub> receptor NAM, MTEP [55]. Interestingly, no tolerance developed to the anti-absence activity of VU0360172, which was still visible after at least 10 days of repeated administrations [54]. We have further explored the mechanism responsible for the anti-absence activity of mGlu<sub>5</sub> receptors by locally administering VU0360172 in the thalamus or somatosensory cortex of WAG/Rij rats. Injection in both anatomical sites resulted in a substantial anti-absence activity. In the cortex, the GABA transporter inhibitor tiagabine acted similar to the PAM, suggesting that the PAM enhanced GABA-ergic neurotransmission. Another interesting finding was obtained when VU0360172 was coinfused with the tiagabine, in the thalamus. Intrathalamic injection of tiagabine alone enhanced the frequency of occurrence of SWDs at doses of 1 or 2 µg/µl but was inactive at the dose of 0.5 µg/µl. However, the inactive dose of tiagabine prevented the anti-absence effect caused by intrathalamic infusion of VU0360172 [56]. This suggested that the anti-absence activity of mGlu<sub>5</sub> receptor activation in the ventrobasal thalamus was mediated by a mechanism that involved GABA transport. We now have evidence that pharmacological activation of mGlu<sub>5</sub> receptors enhances the expression of the high-affinity GABA transporter, GAT-1, in the thalamus (Authors' unpublished observation), and we are currently exploring whether activation of mGlu<sub>5</sub> receptors influences the mechanism of tonic synaptic inhibition, which relies on the availability of extrasynaptic GABA.

Studies performed in genetic mouse models of absence epilepsy do not support the hypothesis that mGlu<sub>1</sub> or mGlu<sub>5</sub> receptors should be activated to restrain absence seizures. Accordingly, treatment with mGlu<sub>1</sub> or mGlu<sub>5</sub> receptor antagonists reduced SWDs in lethargic (lh/lh) mice, which represent an atypical model of absence epilepsy [39,40]. In contrast,

pharmacological blockade of mGlu<sub>5</sub> receptors did not affect SWDs in an acute pharmacological model of absence epilepsy induced by low doses of PTZ in immature rats [57]. Contrasting data may originate from the use of different models and different drugs (for example, orthosteric vs. allosteric ligands).

### 2.2.1. Group II mGlu receptors and convulsive seizures

An increased expression of mGlu<sub>2/3</sub> receptors (presumably mGlu<sub>3</sub> receptors) has been reported in reactive astrocytes in a rat model of temporal lobe epilepsy induced by angular bundle electrical stimulation [34]. Transforming-growth factor-β (TGF-β), a neuroprotective cytokine produced by astrocytes under the control of mGlu<sub>3</sub> receptors [58], was also up-regulated throughout the hippocampus several days after induction of status epilepticus in rats [34]. In humans, an up-regulation of mGlu<sub>2/3</sub> receptors was found in reactive astrocytes of patients with glioneuronal tumors or cortical focal dysplasia, two conditions associated with intractable epilepsy [59,60].

Analysis of mGlu<sub>2</sub> and mGlu<sub>3</sub> receptors in neurons showed a large reduction in the expression and function of both subtypes in the hippocampal dentate gyrus and cortex in the pilocarpine model of chronic epilepsy 24 h after the induction of status epilepticus (SE) [61]. The transcript of mGlu<sub>2</sub> receptors was still down-regulated in the dentate gyrus of pilocarpine-treated rats two months after the onset of status epilepticus, whereas the transcript of mGlu<sub>3</sub> receptors returned back to normal at this time point [62]. This raises the possibility that a long-lasting reduction in the expression and function of pre-synaptic mGlu<sub>2</sub> receptors causes a sustained increase in glutamate release, thereby contributing to epileptogenesis in the hippocampus. In contrast, an up-regulation of mGlu<sub>3</sub> receptors in reactive astrocytes might result in an enhanced production of TGF-β [58] and other neurotrophic factors, thereby protecting neighbor neurons against seizure-induced damage.

Pharmacological data converge to demonstrate protective activity of group-II mGlu receptor agonists against convulsive seizures. Intracerebroventricular infusion of (S)-4-carboxy-3-hydroxyphenylglycine [(S)-4C3HPG], a mixed mGlu<sub>1</sub> receptor antagonist/mGlu<sub>2</sub> receptor agonist, dose-dependently antagonized pentylenetetrazol- and methyl-6,7-dimethoxy-4-ethyl-beta-carboline-2-carboxylate (DMCM)-induced clonic convulsions in mice [63]. (2S,1'R,2'R,3'R)-2-(2,3-dicarboxycyclopropyl) glycine (DCG-IV), a group-II mGlu receptor agonist that also interacts with NMDA receptors, enhanced the threshold for generalized seizures in kindled rats [64], and attenuated kainate-induced limbic seizures [65].

Similar data were obtained by second-generation group-II mGlu receptor agonists, which display high potency, subtype-selectivity, and brain penetration. For example, the mGlu<sub>2/3</sub> receptor agonist, (-)-2-oxa-4-aminobicyclo[3.1.0]hexane-4,6-dicarboxylate (LY379268), reduced the behavioral and electrographic course of acute status epilepticus induced by pilocarpine in mice [66]. LY354740, another potent mGlu<sub>2/3</sub> agonist clinically developed for the treatment of generalized anxiety disorder [67], exerted a strong inhibition of epileptiform activity in rats by reducing the excitability of

entorhinal neurons that are involved in the generation and propagation of epilepsy [68]; in addition, LY354740 enhanced the anticonvulsant effects of diazepam [69]. (2R,4R)-4-Aminopyrrolidine-2,4-dicarboxylate (2R, 4R-APDC), another potent and selective mGlu<sub>2/3</sub> receptor agonist, showed protective effects against pilocarpine-induced status epilepticus in adult rats [70].

Two elegant studies from the same research group [71,72] addressed the important issue of how group-II mGlu receptor ligands interact with classical anticonvulsant drugs using the 6-Hz model of psychomotor seizures in mice. In the first study [71] the Authors were able to demonstrate a pharmacological synergism between levetiracetam and three drugs that activate mGlu<sub>2</sub> receptors: the selective mGlu<sub>2</sub> receptor PAMs, JNJ-42153605 (3-(Cyclopropylmethyl)-7-(4-phenyl-1-piperidinyl)-8-(trifluoromethyl) [1,2,4]triazolo[4,3-a]pyridine) and JNJ-40411813 (1-butyl-3-chloro-4-(4-phenyl-1-piperidinyl)-(1H)-pyridone), and the potent mGlu<sub>2/3</sub> receptor agonist, LY404039 ((1R,4S,5S,6S)-4-amino-2-thiabicyclo[3.1.0]hexane-4,6-dicarboxylic acid 2,2-dioxide). Subthreshold doses of the three mGlu<sub>2</sub> receptor ligands increased the potency of levetiracetam by >25 fold, whereas a moderately effective dose of levetiracetam increased the potency of JNJ-40411813 by more than 10 fold. This likely reflected a pharmacodynamic interaction because plasma levels of levetiracetam and JNJ-40411813 did not differ when the two drugs were administered in combination [71]. In the second study [72], the Authors confirmed the pharmacodynamic synergism between JNJ-46356479 (8-Trifluoromethyl-3-cyclopropylmethyl-7-[(4-(2,4-difluorophenyl)-1-piperazinyl)methyl]-1,2,4-triazolo[4,3-a]pyridine) and levetiracetam in the 6-Hz test with the aid of isobolographic analysis, and could also demonstrate a synergism of the two drugs in the corneal kindling model. Interestingly, there was no synergism between JNJ-40411813 and lamotrigine or valproate, two drugs that share the ability to inhibit voltage-sensitive sodium channels. These studies have a high translational value suggesting that drugs that activate mGlu<sub>2</sub> receptors may lower the required effective dose of levetiracetam, therefore optimizing the risk-to-benefit ratio of levetiracetam in the treatment of temporal lobe epilepsy and perhaps other types of epilepsy. Interestingly, JNJ-40411813 (ADX-71149) is currently being prepared for a Phase 2 clinical study by Addex Therapeutics and Janssen Pharmaceuticals for the treatment of epilepsy.

### 2.2.2. Group-II mGlu receptors in models of absence epilepsy

As opposed to findings obtained in models of convulsive seizures, the role of mGlu<sub>2/3</sub> receptors in models of absence epilepsy is controversial. Using lethargic mice to model absence epilepsy, Brian Meldrum and his Associates have found that treatment with the selective mGlu<sub>2/3</sub> receptor agonists LY379268 and (-)-2-thia-4-aminobicyclo[3.1.0]hexane-4,6-dicarboxylate (LY389795) reduced SWD duration [73].

In contrast, we have found that systemic treatment with LY379268 enhanced, whereas the preferential mGlu<sub>2/3</sub> receptor antagonist, LY341495 ((2S)-2-amino-2-[(1S,2S)-2-carboxycycloprop-1-yl]-3-(xanth-9-yl) propanoic acid), reduced, the number of SWDs in the WAG/Rij rat model [74]. The contrasting findings obtained in two different models of absence

epilepsy [73,74] are difficult to explain. Traditionally, mGlu<sub>2</sub> and mGlu<sub>3</sub> receptors have been considered as presynaptic receptors negatively modulating neurotransmitter release. However, recent findings suggest that mGlu<sub>3</sub> receptors are also found in postsynaptic elements, where they functionally interact with mGlu<sub>5</sub> receptors. Activation of mGlu<sub>3</sub> receptors boost mGlu<sub>5</sub> receptor signaling, and mGlu<sub>3</sub> receptor-dependent long-term depression in the prefrontal cortex requires the endogenous activation of mGlu<sub>5</sub> receptors [12]. It is possible that differences in the expression and/or function of mGlu<sub>2</sub> and mGlu<sub>3</sub> receptors in the cortico-thalamo-cortical network underlying SWDs in lethargic mice, and WAG/Rij rats contributes to the different outcome of treatments with mGlu<sub>2/3</sub> receptor agonists. Experiments with subtype-selective modulators (selective PAMs or NAMs of mGlu<sub>2</sub> and mGlu<sub>3</sub> receptors) should be carried out to unravel the precise role played by either receptor subtypes in the two models of absence epilepsy.

### 2.3. Group-III mGlu receptors and convulsive seizures

For many years, the study of group III mGlu receptors in epilepsy has been hampered by the lack of subtype-selective ligands. More recently, the development of both orthosteric and allosteric ligands targeting individual group-III mGlu receptor subtypes has encouraged investigation of these receptors in CNS disorders, including epilepsy [75]. A role for group-III mGlu receptors in epilepsy is suggested by the evidence that changes in the transcripts of mGlu<sub>4</sub> receptors are found in the hippocampal dentate gyrus of mice developing limbic seizures in response to pilocarpine. Interestingly, increases in mGlu<sub>4</sub> receptor mRNA levels were found in CD1 mice, but not in FVB/N mice, which are more susceptible to pilocarpine-induced seizures. Hence, it has been speculated that mGlu<sub>4</sub> receptors might have a protective role against limbic convulsive seizures in the pilocarpine SE mode [76]. However, this interpretation is not in line with pharmacological data showing that i.c.v. treatments with L-2-amino-4-phosphonobutanoate (L-AP4) and L-serine-O-phosphate (L-SOP) (the two prototypical orthosteric agonists of all group-III mGlu receptor subtypes) are pro-convulsant in both nonepileptic Swiss-Webster and epileptic DBA/2 mice [77]. Curiously, i.c.v. administration of non-subtype-selective group-III mGlu receptors antagonists also produced pro-convulsant effects in these mice [77]. Similar findings were obtained with the mGlu<sub>4</sub> receptor PAM, N-phenyl-7-(hydroxyimino)cyclopropa[b]chromen-1a-carboxamide (PHCCC), in three models of epileptic seizures in immature rats: PHCCC potentiated the effect of a sub-convulsant dose of PTZ, significantly prolonged the duration of PTZ-induced rhythmic activity episodes, and shortened the intervals between individual episodes [78].

In other studies, pharmacological activation of group-III mGlu receptors produced anticonvulsant effects. For example, the group-III mGlu receptor agonist, ACPT-1 ((1S,3R,4S)-1-aminocyclopentane-1,2,4-tricarboxylic acid), reduced DHPG-induced seizures in rats and sound-induced seizures in both DBA/2 mice and genetically epilepsy-prone (GEP) rats [79]. Another group-III agonist, (R,S)-4-phosphonophenylglycine (PPG), displayed anticonvulsant

activity, and its action was abrogated in mice lacking mGlu<sub>7</sub> receptors [80]. (S)-3,4-dicarboxyphenylglycine (DCPG), a selective agonist of mGlu<sub>8</sub> receptors, was able to reduce DL-homocysteic acid-induced seizures and to suppress clonic-tonic seizures [81]. In addition, a down-regulation of mGlu<sub>8</sub> receptors has been detected in pilocarpine-epileptic rats [82]. Clearly, the role played by group-III mGlu receptor subtypes in the regulation of convulsive seizures is not homogeneous, and off-target effects of agonists and antagonists used in the various studies cannot be ruled out. While it appears that mGlu<sub>8</sub> and mGlu<sub>7</sub> receptors display anticonvulsant properties, the role of mGlu<sub>4</sub> receptors in the pathophysiology and control of convulsive seizures remains controversial.

### 2.3.1. Group-III mGlu receptors in models of absence epilepsy

A series of findings combining electrophysiological analysis in thalamic neurons, preclinical models, and genetic studies in humans suggest that mGlu<sub>4</sub> and mGlu<sub>7</sub> receptors are involved in the pathophysiology of absence seizures and can be targeted by therapeutic interventions (reviewed by Ngomba et al. [83]). A pro-absence activity of mGlu<sub>4</sub> receptors is suggested by the evidence that mGlu<sub>4</sub> receptor knockout mice are resistant to absence seizures evoked by low doses of PTZ, whereas local injection of a mGlu<sub>4</sub> receptor agonist in wild-type mice exacerbated pharmacologically induced absence seizures [84]. This hypothesis is supported by our studies in WAG/Rij rats, which showed an increased expression of mGlu<sub>4</sub> receptors in the reticular thalamic nucleus as compared to non-epileptic control rats [85]. In symptomatic WAG/Rij rats, systemic administration of the mGlu<sub>4</sub> receptor PAM, PHCCC, enhanced the incidence of SWDs [85]. Interestingly, polymorphic variants of GRM4, the gene encoding for the mGlu<sub>4</sub> receptor, have been associated with juvenile myoclonic epilepsy (JME), an epileptic syndrome belonging to the group of genetic generalized epilepsies and characterized, *inter alia*, by the occurrence of absence seizures [86–88].

The mGlu<sub>7</sub> receptor may play a central role in the pathophysiology of thalamo-cortical oscillations underlying SWDs and absence seizures. Federica Bertaso and her Associates from the Institute of Functional Genomics in Montpellier (France) have shown that genetic or pharmacological disruption of the mGlu<sub>7</sub> receptor or its PDZ-interacting protein, protein interacting with C kinase 1 (PICK1), causes behavioral and EEG manifestations typical of absence epilepsy [89]. Mice lacking the PDZ domain of mGlu<sub>7</sub> receptors are also more susceptible to absence seizures induced by low doses of PTZ [90]. These findings laid the groundwork for an in-depth study of the role played by mGlu<sub>7</sub> receptors in the pathophysiology of the cortico-thalamo-cortical network. By combining optogenetics, electrophysiological recordings, and pharmacology, Tassin et al. [91] have found that mGlu<sub>7</sub> receptors are presynaptically localized in both glutamatergic and GABAergic terminals, where they inhibit neurotransmitter release and short-term synaptic plasticity. Similar findings were obtained *in vitro* by Kyuyong and Huguenard [92], who found that high concentrations of L-AP4, which likely recruit mGlu<sub>7</sub> receptors at reticular thalamic-ventrobasal thalamic synapses, inhibit pathological oscillations underlying absence seizures. Selective pharmacological blockade of mGlu<sub>7</sub> receptors with the NAM, ADX71743 (6-(2,4-dimethylphenyl)-2-ethyl-6,7-dihydrobenzo[d]

oxazol-4(5H)-one), enhanced synaptic transmission in the thalamus inducing SWDs at the EEG, and behavioral arrest [91]. Taken together, these findings raise the interesting possibility that functional alterations in mGlu<sub>7</sub> receptors cause pathological oscillations within the cortico-thalamo-cortical network underlying absence seizures and that drugs that selectively enhance mGlu<sub>7</sub> receptor function might be therapeutically helpful against absence epilepsy.

## 3. Conclusion

After many years of extensive research, it is frustrating that no mGlu receptor ligands are present in the drug market, as yet. However, many subtype-selective ligands are still under development for the treatment of CNS disorders, and the hope that some of them will be soon available for human use is still alive. A synopsis of studies showing protective effects with mGlu receptor ligands in experimental animal models of epilepsy is shown in Table 1. Synaptic localization and function of individual mGlu receptor subtypes are shown in Figure 1. mGlu<sub>5</sub> receptor NAMs appear to be promising candidates for the treatment of convulsive epileptic syndromes, whereas, in our opinion, mGlu<sub>5</sub> receptor PAMs may enrich the therapeutic armamentarium for the treatment of absence epilepsy. It is more often the case that anti-epileptic drugs have opposite effects on convulsive and non-convulsive epilepsies. A potential drawback in the use of mGlu<sub>5</sub> receptor NAMs is cognitive dysfunction, which might result from an impairment of activity-dependent synaptic plasticity. In contrast, the neuroprotective activity of mGlu<sub>5</sub> NAMs (reviewed by Nicoletti et al. [9]; Bruno et al. [24]) might restrain excitotoxic neuronal death occurring in some epileptic syndrome (e.g., temporal lobe epilepsy associated with Ammon's horn sclerosis). The evidence that mGlu<sub>5</sub> receptors are anatomically and functionally linked to NMDA receptors raises the possibility that a prolonged use of high doses of mGlu<sub>5</sub> NAMs may cause neurodegeneration and psychotomimetic effects [93], which would seriously limit the development of these drugs in the treatment of absence epilepsy. The use of biased mGlu<sub>5</sub> PAMs, which amplify mGlu<sub>5</sub> receptor function without potentiating NMDA receptor activity [94], may help overcome this limitation. The elucidation of the precise mechanisms whereby mGlu<sub>5</sub> receptors restrain pathological oscillation within the cortico-thalamo-cortical network will give the impetus for the development of mGlu<sub>5</sub> receptor PAMs for the treatment of absence epilepsy. Moreover, studies aimed towards antiepileptogenesis in absence models (or in any other model for epileptogenesis), as recently reviewed [95], using mGlu<sub>5</sub> PAMs are still lacking. Preclinical data are still insufficient to conclude that mGlu<sub>2/3</sub> ligands could be promising candidate drug targets for the treatment of convulsive or absence epilepsy. However, the pharmacodynamic synergism between mGlu<sub>2</sub> receptor PAMs and levetiracetam [71,72] holds promise from a therapeutic standpoint. The recent availability of mGlu<sub>2</sub>- and mGlu<sub>3</sub>-selective NAMs will help to elucidate the precise role of the two receptor subtypes in the pathophysiology of epilepsy [96–99].

In contrast, mGlu<sub>7</sub> receptors appear to be tightly linked to the pathophysiology of absence epilepsy (at least in animal models), and, this encourages the development of selective mGlu<sub>7</sub> receptor agonists or PAMs for the treatment of this disorder.

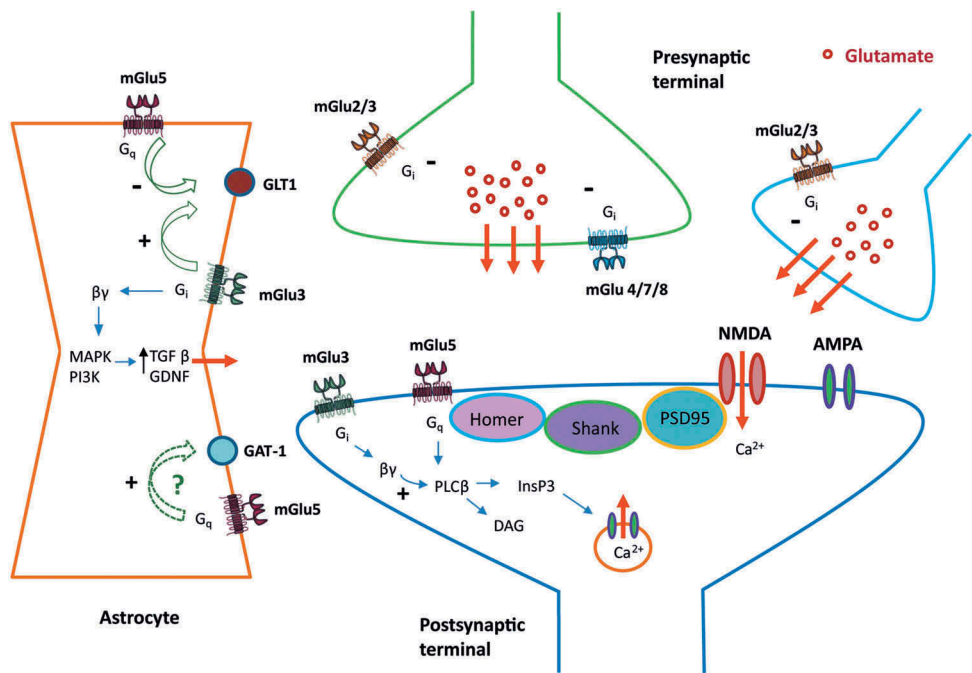
**Table 1.** Synopsis of studies showing protection with mGlu receptor ligands in experimental animal models of epilepsy.

Subtype	Ligand	Treatment	Animal model	Ref.
mGlu <sub>1</sub>	LY367385 (Ant)	i.c.v.	Audiogenic seizures in DBA/2 mice	39
	AIDA (Ant)	IColl infusion	Spontaneous SWDs in <i>lethargic</i> mice	39
	LY367385 (Ant)	i.c.v.	Audiogenic seizures in GEPRs	42
	AIDA (Ant)	i.p., 1.5 mg/kg	DHPG-induced seizures	44
	LY367385 (Ant)	i.c.v.	Audiogenic seizures in GEPRs	45
	LY339840 (Ant)	s.c., 3 or 10 mg/kg	Diazepam withdrawal	51,54
	AIDA (Ant)	single injection only	WAG/Rij rats	52
	4CPG (mGlu <sub>1/5</sub> Ant)	IT infusion	WAG/Rij rats	
	RO0711401 (PAM)			
	DHPG (mGlu <sub>1/5</sub> Ago)			
mGlu <sub>5</sub>	MPEP (NAM)	i.c.v. or i.p., multiple doses	Audiogenic seizures in DBA/2 mice	40
	SIB-1893 (NAM)		Hippocampal stimulation	43
	MTEP (NAM)	i.p., 20 or 40 mg/kg	Audiogenic seizures in GEPRs	44
	MPEP (NAM)	i.p., 10 mg/kg	WAG/Rij rats	54,55
	VU0360172 (PAM)	s.c., 3 or 10 mg/kg single and repeated injections	<i>lethargic</i> mice	56
	MPEP (NAM)	ICtx and IT infusions		40
mGlu <sub>2/3</sub>		i.p., 50 mg/kg		
	4C3HPG (Ago)	i.c.v.	Clonic seizures by PTZ or DMCM	63
	DCG-IV (Ago)	i.c.v.	Amygdaloid kindling	64
	DCG-IV (Ago)	i.c.v.	Kainate-induced seizures	65
	LY379268 (Ago)	i.p., 10 mg/kg	Pilocarpine-induced SE	66
	LY354740 (Ago)	i.p., 4–16 mg/kg	Picrotoxin- and PTZ-induced seizures	69
	2R,4R-APDC (Ago)	i.c.v.	Pilocarpine-induced SE	70
	LY389795 (Ago)	i.c.v. or i.p.	DBA/2 mice, amygdaloid kindling	73
	LY379268 (Ago)	both at multiple doses	GEPRs, <i>lethargic</i> mice	71,72
	LY404039 (Ago)	s.c., multiple doses	6-Hz model and corneal kindling	74
	JNJ-42153605 (mGlu <sub>2</sub> PAM)	s.c., multiple doses		
	JNJ-40411813 (mGlu <sub>2</sub> PAM)	s.c., multiple doses		
	JNJ-46356479	s.c., multiple doses		
	LY341495 (Ant)	i.p., multiple doses		
mGlu <sub>4/7/8</sub>	ACTP-1 (mGlu <sub>4</sub> Ago)	i.c.v.	DBA/2 mice and GEPRs	79
	(R,S)-PPG (Ago)	i.c.v.	PTZ in mice (lost in mGlu <sub>7</sub> <sup>-/-</sup> mice)	80
	(S)-DCPG (mGlu <sub>8</sub> Ago)	i.c.v.	L-Homocysteic-induced seizures	81

Only studies showing protection against seizures with mGlu receptor ligands are reported. For studies showing no effect or aggravation of seizures, see the appropriate sections in the text.

Ago = orthosteric agonist; Ant = orthosteric antagonist; i.c.v. = intracerebroventricular infusion; i.p. = intraperitoneal injection; IColl infusion = infusion in the inferior colliculus; ICtx infusion = intracortical infusion; IT infusion = intrathalamic infusion; NAM = negative allosteric modulator; PAM = positive allosteric modulator; s.c. = subcutaneous administration.

For abbreviations and molecular structures of mGlu receptor ligands and other drugs, see appropriate sections in the text.



**Figure 1.** Synaptic localization and function of individual mGlu receptor subtypes.

Negative modulation of glutamate release by mGlu<sub>2</sub> receptors contributes to explain the encouraging data obtained with mGlu<sub>2</sub> receptor PAMs in models of convulsive seizures [71,72]. mGlu<sub>5</sub> receptor NAMs may restrain convulsive seizures by inhibiting postsynaptic mGlu<sub>5</sub> receptors, which are functionally linked to NMDA receptors (see main text). Positive modulation of GAT-1 receptors by mGlu<sub>5</sub> receptors PAMs is hypothetical and contributes to explain the protective activity of mGlu<sub>5</sub> receptor PAMs in the WAG/Rij rat model of absence epilepsy (see main text). For a more detailed representation of the role played by individual mGlu receptor subtypes in the regulation of the cortico-thalamo-cortical network underlying absence seizures, see Ref [83]. and [101].



#### 4. Expert opinion

Although glutamate is a main player of over-excitation in epilepsy, glutamate receptor antagonists were not successfully developed as antiepileptic drugs, with the notable exception of perampanel. One of the reasons is that iGlu receptor antagonists are not disease-dependent and cause a widespread inhibition of excitatory synaptic transmission in the CNS. In addition, NMDA receptor antagonists impair mechanisms of activity-dependent synaptic plasticity and may cause psychotomimetic effects. Targeting mGlu receptors with subtype-selective ligands (particularly, PAMs and NAMs) might offer a more favorable outcome in the treatment of epilepsy because mGlu receptors do not mediate, but rather modulate, synaptic transmission. Using these drugs, we expect a greater selectivity for overexcited neuronal networks (for example, the action of PAMs is activity-dependent), and a better profile of safety and tolerability.

Metabotropic glutamate (mGlu) receptors were discovered in 1985. Since then, they have attracted the interest of neuroscientists in many different fields with great potential as candidate drug targets for the treatment of CNS disorders. Preclinical data and clinical studies carried out in CNS disorders other than epilepsy indicate, in general, a good profile of safety and tolerability for most mGlu receptor ligands, and this strengthens the potential value of these drugs in the treatment of epilepsy.

Drugs that inhibit mGlu<sub>1</sub> and mGlu<sub>5</sub> receptors (either NAMs or orthosteric antagonists) have consistently shown protective activity in models of convulsive epilepsy. However, the potential detrimental effects of mGlu<sub>1</sub> receptor antagonists on cerebellar function [100] and the impact of mGlu<sub>5</sub> NAMs on mechanisms of activity-dependent synaptic plasticity (see above) may limit the use of these drugs in the treatment of epilepsy. The exciting data obtained with the 6-Hz model of psychomotor seizures [71,72] suggest that mGlu<sub>2</sub> receptor PAMs are promising candidate drugs for the treatment of focal epilepsy and may act synergistically with levetiracetam, one of the most widely used drugs in epilepsy. Although the molecular nature of this pharmacodynamics interaction is unknown, these findings are highly promising from a therapeutic standpoint.

Our data obtained in WAG/Rij rats (a well-developed and validated model of absence epilepsy) encourage the development of mGlu<sub>5</sub> PAMs for the treatment of absence seizures. These drugs might act by enhancing GABA reuptake in ventrobasal thalamic nuclei. These drugs suppress SWDs both in cortex and in thalamus. In the cortex by stimulating GABA-ergic interneurons, in the ventrobasal thalamic nuclei by enhancing GABA-ergic reuptake. mGlu<sub>5</sub> PAMs are under development for the treatment of schizophrenia and display cognitive enhancing effects in rodents. Stimulus-biased mGlu<sub>5</sub> receptor PAMs that do not activate NMDA receptors show no neurotoxic effects in rodents and are particularly promising from a therapeutic standpoint. We predict that drugs that selectively activate mGlu<sub>7</sub> receptors are effective in the treatment of absence epilepsy. Both mGlu<sub>5</sub> and mGlu<sub>7</sub> PAMs might be clinically helpful in patients that are refractory to conventional medications and require

polytherapy for an adequate control of absence seizures. Thus, the study of mGlu receptors in epilepsy represents an exciting example of translational research that holds promise for the treatment of one of the most common CNS disorders which is a high social and economic burden worldwide.

A dark side is that the role of mGlu receptors in mechanisms of epileptogenesis is lacking, and, in general, most of the existing data were obtained after acute or temporally restricted administration of mGlu receptor ligands, whereas drug treatment of epilepsy in humans usually lasts for several months or years (except for emergency treatment of SE). It will be extremely interesting to examine the effect of mGlu receptor ligands on epileptogenesis in genetic and kindling models of epilepsy, and to investigate whether chronic treatments with these drugs restrains the development or recurrence of seizures (for example, by treating pre-symptomatic WAG/Rij rats with mGlu<sub>5</sub> receptor PAMs or by administering appropriate receptor ligands after SE during the so-called 'silent' period in the pilocarpine and kainic acid models).

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#### Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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